

U.S.S.N. 07/938,154

HARPOLD, *et al.*

SECOND SUBMISSION UNDER RULE 129(a)

IN THE CLAIMS:

Please cancel claim 57, 58, 61, ~~63~~, 67, 71, 73, 74, 76-78, 86-90 and 99 without prejudice or disclaimer. 1-28-98

Please add claims 100-112, which replace and amend cancelled claims as follows:

100. An isolated nucleic acid, comprising the sequence of nucleotides set forth in SEQ ID No. 1.—

101. An isolated nucleic acid, comprising the sequence of nucleotides set forth in SEQ ID No. 3.—

102. An isolated nucleic acid, comprising the sequence of nucleotides set forth in SEQ ID No. 5.—

103. An isolated nucleic acid, comprising the sequence of nucleotides set forth in SEQ ID No. 7.—

104. An isolated nucleic acid molecule encoding a beta2 subunit of a human neuronal nicotinic acetylcholine receptor, comprising the sequence of nucleotides set forth in SEQ ID No. 9.—

105. A plasmid having all of the identifying characteristics of the plasmid deposited under ATCC Accession No. 68278.—

106. A plasmid having all of the identifying characteristics of the plasmid deposited under ATCC Accession No. 68279.—

107. The cell of claim 105 that is a bacterial or eukaryotic cell.—

108. The cell of claim 106 that is a mammalian cell, yeast cell or amphibian oocyte.—

109. An isolated cell, comprising the nucleic acid of claim 100.—

110. The cell of claim 109 that is a bacterial or eukaryotic cell.—

111. The cell of claim 110 that is a mammalian cell, yeast cell or amphibian oocyte.—

112. The cell of claim 111 that expresses a nicotinic acetylcholine receptor comprising a subunit encoded by the nucleic acid.—

47

U.S.S.N. 07/938,154
HARPOLD, *et al.*
SECOND SUBMISSION UNDER RULE 129(a)

Please amend claims 53, 55, 56, 59, 60, 62, 63, 66, 68, 70, 72, 82-84, 91, 92 and 98 as follows:

#2
53. (Amended) An isolated nucleic acid molecule, comprising a sequence of nucleotides encoding [an alpha2 subunit of a human nicotinic acetylcholine receptor that is encoded by nucleic acid comprising the sequence of nucleotides set forth SEQ ID No. 1 and 3, an alpha3 subunit of a human nicotinic acetylcholine receptor that is encoded by nucleic acid comprising the sequence of nucleotides set forth in SEQ ID No. 5 and 7 or] a beta2 subunit of a human neuronal nicotinic acetylcholine receptor[that is encoded by nucleic acid comprising the sequence of nucleotides set forth in SEQ ID No. 9].

#3
55. (Amended) [A] An isolated and purified [substantially pure subunit of the] human neuronal nicotinic acetylcholine receptor subunit encoded by the alpha3-encoding nucleic acid in a plasmid having all of the identifying characteristics of HnAChR α 3 deposited under ATCC Accession No. 68278.

56. (Amended) [A] An isolated and purified [substantially pure subunit of the] human neuronal nicotinic acetylcholine receptor subunit encoded by the beta2-encoding nucleic acid [of claim 53] in a plasmid having all of the identifying characteristics of HnAChR α 3 deposited under ATCC Accession No. 68279.

#4
59. (Amended) An isolated cell, comprising [containing any one or more of] the nucleic [acids of claim 53] acid molecule of claim 80.

60. (Amended) An isolated cell, comprising [containing one or more of] the nucleic [acids of claim 54] acid molecule of claim 81.

#5
62. (Amended) The cell of claim [59] 80 that is a eukaryotic cell.

#6
63. The cell of claim 59 that is a bacterial cell, mammalian cell, yeast cell or amphibian oöcyte.

#6
66. The cell of claim 59 further comprising a nucleic acid molecule that encodes a beta subunit of a human nicotinic acetylcholine receptor, wherein the beta subunit comprises a sequence of amino acids encoded by SEQ ID No. 9.

H7 1) ¹⁰68. (Twice amended) A method for screening compounds for activity as nicotinic acetylcholine receptor agonists or antagonists, said method comprising:

I contacting [cells] a cell of claim 59 with a test compound, and thereafter monitoring nicotinic acetylcholine receptor activity of the cells by monitoring the performance of the [cells] cell by measuring a performance parameter selected from the group consisting of the flux of ions through the [membranes] membrane of the [cells] cell, nicotine binding to nicotinic acetylcholine receptors of the cell, or the electrophysiological response of the cells, wherein[:

the cells contain one or more nucleic acids comprising a sequence of nucleotides (i) encoding an alpha2 subunit of a human nicotinic acetylcholine receptor and comprising a sequence of nucleotides set forth SEQ ID No. 1 and 3, (ii) encoding an alpha3 subunit of a human nicotinic acetylcholine receptor and comprising a sequence of nucleotides set forth in SEQ ID No. 5 and 7, or (iii) encoding a beta2 subunit of a human neuronal nicotinic acetylcholine receptor and comprising a sequence of nucleotides set forth in SEQ ID No. 9; and] the [cells express] cell expresses a nicotinic acetylcholine receptor that contains a [one or more subunits] subunit encoded by the nucleic [acids] acid molecule.

H8 1) ¹⁰70. (Amended) The method of claim ¹⁰68, wherein ~~the~~ [alpha subunit is an alpha2 subunit encoded by a sequence of nucleotides having the restriction map of the DNA encoding the human alpha2 subunit set forth in Figure 1 or an alpha3 subunit encoded by a sequence of nucleotides having the restriction map of the DNA encoding the human alpha3 subunit set forth in Figure 2, and the beta subunit is encoded by a sequence of nucleotides having the restriction map of the DNA encoding the human beta2 subunit set forth in Figure 3] the cell further comprises DNA encoding a beta2 subunit of a human nicotinic

U.S.S.N. 07/938,154

HARPOLD, et al.

SECOND SUBMISSION UNDER RULE 129(a)

H8. crit
acetylcholine receptor comprising a sequence of amino acids encoded by SEQ ID No.9.

H9
L1 72. (Amended) A method of making cells having neuronal nicotinic acetylcholine receptor activity, comprising:

(a) introducing one or more nucleic acid molecules that encode(s) at least one alpha subunit of a neuronal nicotinic acetylcholine receptor and at least one beta subunit of a neuronal nicotinic acetylcholine receptor ^{into} eukaryotic cells, wherein the nucleic acid encoding an $[\alpha]$ alpha subunit comprises [the] a sequence of amino acids encoded by [SEQ ID No. 1 or 3, or comprises the sequence of amino acids encoded by SEQ ID No. 5 or 7,] the alpha3-encoding nucleic acid that is isolated from a plasmid having all of the identifying characteristics of HnAChR α 3 deposited under ATCC Accession No. 68278, and the nucleic acid encoding the beta subunit comprises [the] a sequence of amino acids encoded by SEQ ID No. 9;

(b) selecting cells from (a) that express the alpha or the beta encoding nucleic acid or express the alpha and beta subunit-encoding nucleic acid; and

(c) detecting neuronal nicotinic acetylcholine receptor activity in the selected cells, wherein the activity is mediated by a receptor containing one or more of the alpha and beta subunits encoded by said introduced nucleic acid molecules.

H10
79. (Amended) An isolated [Isolated] nucleic acid[of claim 57] molecule, comprising the alpha2-encoding nucleic acid open reading frame that is isolated from a plasmid having all of the identifying characteristics of HnAChR α 2 deposited under ATCC Accession No. 68277.

80. (Amended) [Isolated nucleic acid of claim 57, comprising] An isolated nucleic acid molecule, comprising a sequence of nucleotides encoding an alpha3 subunit of a human nicotinic acetylcholine receptor that is encoded by the alpha3-encoding nucleic acid that is isolated from a plasmid having all of the

U.S.S.N. 07/938,154

HARPOLD, *et al.*

SECOND SUBMISSION UNDER RULE 129(a)

H10
cont
70 81. identifying characteristics of HnAChR α 3 deposited under ATCC Accession No. 68278.

70 81. (Amended) [Isolated] An isolated nucleic acid molecule, [of claim 57] comprising a sequence of nucleotides encoding a beta2 subunit of a human nicotinic acetylcholine receptor that is encoded by the beta2-encoding nucleic acid that is isolated from a plasmid having all of the identifying characteristics of HnAChR β 2 deposited under ATCC Accession No. 68279 or the sequence of nucleotides set forth as nucleotides 1-1521 in SEQ ID No. 9.

H11
Pach II
82. (Amended) An isolated and purified protein[subunit of a human nicotinic acetylcholine receptor] encoded by the nucleic acid of claim 79.

83. (Amended) An isolated and purified subunit of a human nicotinic acetylcholine receptor encoded by the nucleic acid of claim 80.

84. (Amended) An isolated and purified subunit of a human nicotinic acetylcholine receptor encoded by the nucleic acid of claim 81.

H12
75 81. (Amended) The cell of claim [61] ~~59~~ further comprising [that additionally contains] a reporter gene expression construct; and

the reporter gene expression construct comprises:

a transcriptional control element, and

a reporter gene encoding a transcriptional and/or translational product;

the transcriptional control element, in said cell, is responsive to an intracellular condition that occurs when a human neuronal nicotinic acetylcholine receptor interacts with a compound having agonist or antagonist activity with respect to said receptor;

said product can be, directly or indirectly, detected; and

the reporter gene is in operative association with said transcriptional control element.

U.S.S.N. 07/938,154

HARPOLD, *et al.*

SECOND SUBMISSION UNDER RULE 129(a)

92. (Amended) A method for screening test compounds for activity as nicotinic acetylcholine receptor agonists or antagonists, comprising:

comparing the difference in the amount of transcription of a reporter gene in the cells of claim 91 in the presence of the compound with the amount of transcription in the absence of the compound or with the amount of transcription in the control cells that do not express nicotinic acetylcholine receptors, but contain the reporter gene expression construct, wherein compounds that exhibit activity as agonists or antagonists are identified[, wherein:

the cells contain one or more nucleic acids comprising a sequence of nucleotides (i) encoding an alpha2 subunit of a human nicotinic acetylcholine receptor and comprising a sequence of nucleotides set forth SEQ ID No. 1 and 3, (ii) encoding an alpha3 subunit of a human nicotinic acetylcholine receptor and comprising a sequence of nucleotides set forth in SEQ ID No. 5 and 7, or (iii) encoding a beta2 subunit of a human neuronal nicotinic acetylcholine receptor and comprising a sequence of nucleotides set forth in SEQ ID No. 9;

the cells also contain a reporter gene expression construct; the reporter gene expression construct comprises:

- a transcriptional control element, and
- a reporter gene encoding a transcriptional and/or translational product;

the transcriptional control element, in said cell, is responsive to an intracellular condition that occurs when a human neuronal nicotinic acetylcholine receptor interacts with a compound having agonist or antagonist activity with respect to said receptor;

said product can be, directly or indirectly, detected; and

the reporter gene is in operative association with said transcriptional control element; and

the cells express a nicotinic acetylcholine receptor that contains one or more subunits encoded by the nucleic acids].

52